PATHOGEN SOLUTIONS

Third Party Studies

- NSF International - Applied Research Center
- CREM CO. Laboratory - Coronavirus
- CREM CO. Laboratory - Norovirus
- Microchem Laboratory

NSF International – Applied Research Center
789 N. Dixboro Rd. Ann Arbor, MI 48015, USA
1-800.NSF.MARK | +1-734.769.8010 | www.nsf.org

Send to: Scott Beal
Hepco Medical, LLC
200 Central Ave Ste 2200
St. Petersburg, FL 33701

Result: COMPLETE **Report Date: August 23, 2019**

Customer Name:	Hepco Medical, LLC
Description:	Efficacy of an Ozone-Generating Whole-Shoe Disinfection Device at Three Time Points
Test Type:	Test Only
Job Number:	J-00340388
Project Number:	10120011
NSF Corporate:	C0484938
Project Manager:	S. Hatt

Executive Summary: An efficacy study was performed using a UV-C and ozone-generating device against *Escherichia coli, Pseudomonas aeruginosa*, Methicillin-resistant *Staphylococcus aureus*, Vancomycin-resistant *Enterococcus faecalis*, Carbapenem-resistant *Klebsiella pneumoniae*, *Candida auris*, *Aspergillus brasiliensis*, and *Clostridioides difficile*. Log and percent reduction were quantified for each microorganism at three exposure times: 6, 8, and 10 seconds.

Thank you for having your product tested by NSF International.

Please contact your Project Manager if you have any questions or concerns pertaining to this report.

Report Authorization: Jesse D. Miller

Jesse Miller – Director, Applied Research Center

Written NSF approval is required for reproduction of this report. Only authorized reports in their entirety may be distributed. This report does not represent authorization to use the NSF Mark. NSF Certification may be confirmed at www.nsf.org. The results of this report relate only to those items tested.

Experimental Summary:

Challenge microorganisms:

- *Escherichia coli* ATCC 11229
- *Pseudomonas aeruginosa* ATCC BAA- 2108
- Methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 33592
- Vancomycin-resistant *Enterococcus faecalis* (VRE) ATCC 51299
- Carbapenem-resistant *Klebsiella pneumoniae* (CRE) ATCC BAA-1705
- *Candida auris* CDC B11903
- *Aspergillus brasiliensis* ATCC 16404
- *Clostridioides difficile* ATCC 43598

Test Product:

- PathO_3Gen Solutions™ Footwear Sanitizing Station

Culture Preparation:

- Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant *Enterococcus faecalis* (VRE), *Klebsiella pneumoniae* (CRE), and *Escherichia coli* were propagated onto Tryptic Soy Agar with 5% Sheep Blood (SBA) and were incubated at 35 ± 2°C for 24 ± 2 hours.
- *Candida auris* was propagated onto SBA for 18-24 hours at 25 ± 1°C.
    - Daily transfers were performed using Sabouraud Dextrose Agar with Letheen (SDA/L). Each daily transfer was incubated at the appropriate temperature for growth for 24 ± 2 hours.
    - After incubation, an isolated colony was picked to SDA/L and incubated at 35 ± 2°C for 24 ± 2 hours.
- *Aspergillus brasiliensis* was propogated on SDA/L for 5 to 7 days. After incubation, the culture was washed with 0.85% saline with tween and filtered with through sterile gauze. The culture was centrifuged at 4,500 rpm, the supernatant removed, and the pellet was rehydrated with Phosphate Buffered Saline (PBS).
- *Clostridium difficile* spore suspension was prepared using a modification of the U.S. EPA OPP: MB-28 (December 2017) Procedure for the Production and Storage of Spores of Clostridium difficile for Use in the Efficacy Evaluation of Antimicrobial Agents based on ASTM Standard E2839-11.

Inoculation:

- Hard rubber carriers of approximately 2" x 2" were sterilized prior to testing.
- The soles of the shoe carriers were inoculated with 0.1 mL aliquot of standardized suspension of the challenge microorganism and allowed to dry for 60 ± 5 minutes.

Exposure Period:

- The disinfection device was sterilized using isopropyl alcohol prior to testing.
- After drying, the sole of the shoe carrier was aseptically placed inoculum side down onto the floor disinfection device using sterilized forceps.
- A volunteer (~150 lb) stood on the shoe carrier (with a sterile barrier between the individual and shoe carrier) for the exposure time. The instrument automatically shut off after the exposure time.
- After the exposure time, the shoe carrier was moved to a saline solution using sterile forceps. Three carriers were tested per each microorganism.
    - For bacteria, dilutions were plated via pour plate method in duplicate on Microbial Content Test Agar and incubated for 48 ± 3 hours at 35 ± 2 °C
    - For fungi, dilutions were plated via pour plate method in duplicate on Sabouraud Dextrose Agar with Letheen and incubated for 5 to 7 days at 25 ± 2 °C
    - For spores, dilutions were plated via spread plate method in duplicate on Brucella Blood Agar and incubated for 48 ± 3 hours at 36 ± 2 °C.
- After incubation, colonies were counted, and data recorded. Geometric mean was calculated from the duplicated plates and log and percent reduction were calculated using the positive control counts.

Written NSF approval is required for reproduction of this report. Only authorized reports in their entirety may be distributed. This report does not represent authorization to use the NSF Mark. NSF Certification may be confirmed at www.nsf.org. The results of this report relate only to those items tested.

Results

Written NSF approval is required for reproduction of this report. Only authorized reports in their entirety may be distributed. This report does not represent authorization to use the NSF Mark. NSF Certification may be confirmed at www.nsf.org. The results of this report relate only to those items tested.

Table 1. Geometric mean of the inoculum concentrations on unexposed carriers used for each microorganism. The results shown are the geomean of the inoculum plated in triplicate.

Organism	Time Point	CFU/mL	Log (CFU/mL)
E. coli ATCC 11229	6 seconds	3.03E+07	7.4814
	8 seconds	59000000	7.7706
	10 seconds	3.26E+07	7.5129
Pseudomonas aeruginosa ATCC BAA- 2108	6 seconds	1.52E+06	6.1829
	8 seconds	2.21E+06	6.3439
	10 seconds	1.60E+06	6.2037
Methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 33592	6 seconds	2.79E+07	7.4461
	8 seconds	4.36E+07	7.6398
	10 seconds	4.01E+07	7.6030
Vancomycin-resistant *Enterococcus faecalis* (VRE) ATCC 51299	6 seconds	7.12E+07	7.8528
	8 seconds	7.40E+07	7.8690
	10 seconds	4.64E+07	7.6664
Klebsiella pneumoniae CRE ATCC BAA-1705	6 seconds	1.97E+08	8.2944
	8 seconds	3.36E+08	8.5262
	10 seconds	2.69E+08	8.4302
Candida auris CDC B11903	6 seconds	5.16E+06	6.7129
	8 seconds	1.32E+06	6.1219
	10 seconds	1.86E+06	6.2692
Aspergillus brasiliensis ATCC 16404	6 seconds	6.70E+06	6.8261
	8 seconds	8.41E+06	6.9246
	10 seconds	1.39E+07	7.1438
Clostridioides difficile ATCC 43598	6 seconds	1.15E+07	7.0614
	8 seconds	1.37E+07	7.1380
	10 seconds	1.24E+07	7.0927

Written NSF approval is required for reproduction of this report. Only authorized reports in their entirety may be distributed. This report does not represent authorization to use the NSF Mark. NSF Certification may be confirmed at www.nsf.org. The results of this report relate only to those items tested.

Table 2. Carrier density for each of the carriers inoculated with *E. coli* and exposed to the disinfection device. The results shown are the geomean of each of the carriers, which were plated in triplicate.

Organism	Time Point	Replicate A (CFU/mL)	Replicate B (CFU/mL)	Replicate C (CFU/mL)	Geomean (CFU/mL)	Log CFU/mL	Percent Reduction	Log Reduction
E. coli ATCC 11229	6 seconds	3.96E+05	3.59E+05	1.29E+05	2.64E+05	5.4211	99.1297%	2.06
	8 seconds	2.95E+04	4.14E+04	3.51E+03	1.62E+04	4.2107	99.9725%	3.56
	10 seconds	1.23E+02	4.11E+02	9.23E+02	3.60E+02	2.5563	99.9989%	4.96

Table 3. Carrier density for each of the carriers inoculated with *Pseudomonas aeruginosa* and exposed to the disinfection device. The results shown are the geomean of each of the carriers, which were plated in triplicate.

Organism	Time Point	Replicate A (CFU/mL)	Replicate B (CFU/mL)	Replicate C (CFU/mL)	Geomean (CFU/mL)	Log CFU/mL	Percent Reduction	Log Reduction
Pseudomonas aeruginosa ATCC BAA- 2108	6 seconds	1.89E+03	2.84E+03	3.29E+03	2.60E+03	3.4157	99.8287%	2.77
	8 seconds	4.32E+01	5.15E+01	6.53E+01	5.26E+01	1.7207	99.9976%	4.62
	10 seconds	5.90E+01	6.39E+01	2.82E+01	4.74E+01	1.6755	99.9970%	4.53

Table 4. Carrier density for each of the carriers inoculated with MRSA and exposed to the disinfection device. The results shown are the geomean of each of the carriers, which were plated in triplicate.

Organism	Time Point	Replicate A (CFU/mL)	Replicate B (CFU/mL)	Replicate C (CFU/mL)	Geomean (CFU/mL)	Log CFU/mL	Percent Reduction	Log Reduction
Methicillin Resistant *Staphylococcus aureus* (MRSA) ATCC 33592	6 seconds	2.73E+04	5.25E+04	3.75E+04	3.77E+04	4.5768	99.8647%	2.87
	8 seconds	4.90E+03	5.78E+03	1.15E+04	6.88E+03	3.8376	99.9842%	3.80
	10 seconds	5.18E+02	1.15E+03	3.30E+03	1.25E+03	3.0978	99.9969%	4.51

Table 5. Carrier density for each of the carriers inoculated with VRE and exposed to the disinfection device. The results shown are the geomean of each of the carriers, which were plated in triplicate.

Organism	Time Point	Replicate A (CFU/mL)	Replicate B (CFU/mL)	Replicate C (CFU/mL)	Geomean (CFU/mL)	Log CFU/mL	Percent Reduction	Log Reduction
Enterococcus faecalis VRE ATCC 51299	6 seconds	1.08E+06	1.16E+06	8.14E+05	1.01E+06	6.0028	98.5863%	1.85
	8 seconds	5.83E+03	1.28E+04	1.29E+04	9.87E+03	3.9945	99.9867%	3.87
	10 seconds	7.62E+03	5.88E+03	5.77E+03	6.37E+03	3.8042	99.9863%	3.86

Written NSF approval is required for reproduction of this report. Only authorized reports in their entirety may be distributed. This report does not represent authorization to use the NSF Mark. NSF Certification may be confirmed at www.nsf.org. The results of this report relate only to those items tested.

Table 6. Carrier density for each of the carriers inoculated with CRE and exposed to the disinfection device. The results shown are the geomean of each of the carriers, which were plated in triplicate.

Organism	Time Point	Replicate A (CFU/mL)	Replicate B (CFU/mL)	Replicate C (CFU/mL)	Geomean (CFU/mL)	Log CFU/mL	Percent Reduction	Log Reduction
Klebsiella pneumoniae CRE ATCC BAA-1705	6 seconds	7.26E+05	3.86E+05	1.29E+06	7.12E+05	5.8527	99.6384%	2.44
	8 seconds	8.75E+04	1.20E+05	2.72E+05	1.42E+05	5.1519	99.9578%	3.37
	10 seconds	3.91E+03	1.32E+03	9.85E+03	3.70E+03	3.5687	99.9986%	4.86

Table 7. Carrier density for each of the carriers inoculated with *Candida auris* and exposed to the disinfection device. The results shown are the geomean of each of the carriers, which were plated in triplicate. For plate count geomeans below 10 CFU/mL were input as 10 to calculate percent and log reduction.

Organism	Time Point	Replicate A (CFU/mL)	Replicate B (CFU/mL)	Replicate C (CFU/mL)	Geomean (CFU/mL)	Log CFU/mL	Percent Reduction	Log Reduction
Candida auris CDC B11903	6 seconds	1.09E+03	1.82E+02	6.08E+02	4.94E+02	2.6938	99.9904%	4.02
	8 seconds	1.46E+02	<1.00E+01	2.93E+01	3.50E+01	1.5437	99.9974%	4.58
	10 seconds	2.11E+01	<1.00E+01	<1.00E+01	1.28E+01	1.1081	99.9993%	5.16

Table 8. Carrier density for each of the carriers inoculated with *Aspergillus brasiliensis* and exposed to the disinfection device. The results shown are the geomean of each of the carriers, which were plated in triplicate.

Organism	Time Point	Replicate A (CFU/mL)	Replicate B (CFU/mL)	Replicate C (CFU/mL)	Geomean (CFU/mL)	Log CFU/mL	Percent Reduction	Log Reduction
Aspergillus brasiliensis ATCC 16404	6 seconds	1.18E+06	1.13E+06	1.08E+06	1.13E+06	6.0528	83.1453%	0.77
	8 seconds	5.29E+05	1.00E+06	1.16E+06	8.50E+05	5.9293	89.8956%	1.00
	10 seconds	3.69E+05	1.25E+06	3.26E+05	5.32E+05	5.7257	96.1744%	1.42

Table 9. Carrier density for each of the carriers inoculated with *C. difficile* and exposed to the disinfection device. The results shown are the geomean of each of the carriers, which were plated in triplicate.

Organism	Time Point	Replicate A (CFU/mL)	Replicate B (CFU/mL)	Replicate C (CFU/mL)	Geomean (CFU/mL)	Log CFU/mL	Percent Reduction	Log Reduction
Clostridioides difficile ATCC 43598	6 seconds	1.21E+05	8.61E+04	1.87E+05	1.25E+05	5.0965	98.9140%	1.96
	8 seconds	9.65E+03	9.62E+03	4.86E+03	7.67E+03	3.8848	99.9440%	3.25
	10 seconds	2.26E+03	1.47E+03	3.63E+01	4.94E+02	2.6938	99.9960%	4.40

Written NSF approval is required for reproduction of this report. Only authorized reports in their entirety may be distributed. This report does not represent authorization to use the NSF Mark. NSF Certification may be confirmed at www.nsf.org. The results of this report relate only to those items tested.

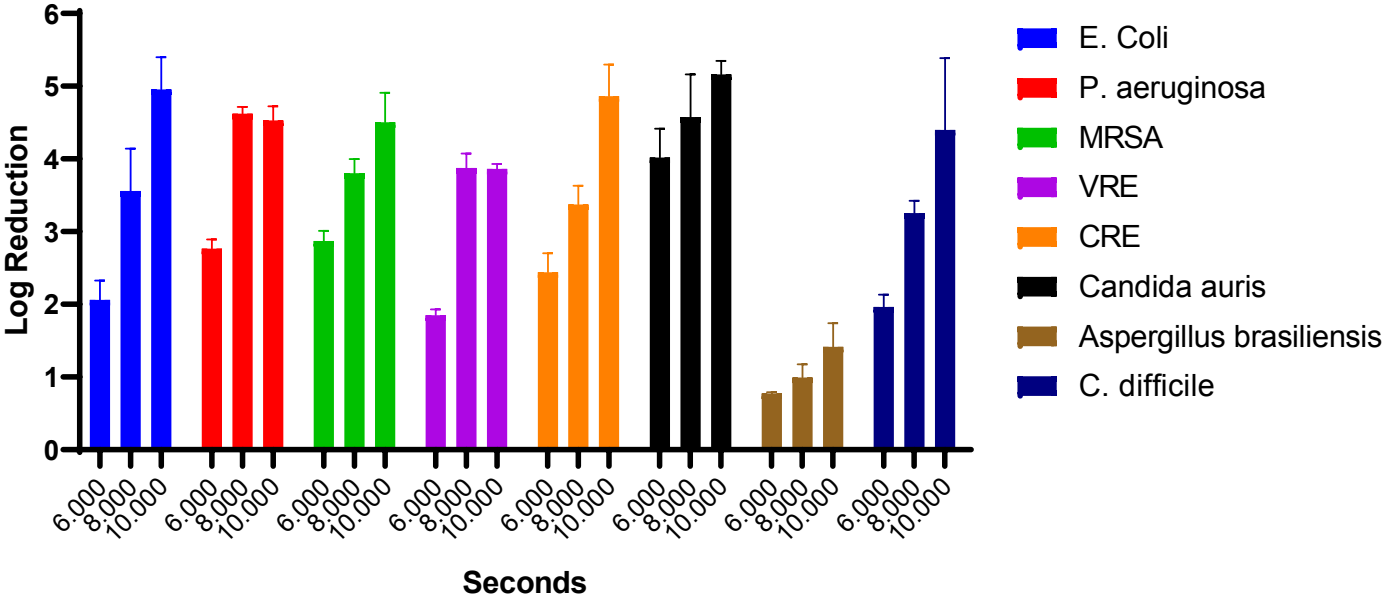


Figure 1. Summary bar plot of mean log reduction at each time point (in seconds) by microorganism tested.

Written NSF approval is required for reproduction of this report. Only authorized reports in their entirety may be distributed. This report does not represent authorization to use the NSF Mark. NSF Certification may be confirmed at www.nsf.org. The results of this report relate only to those items tested.

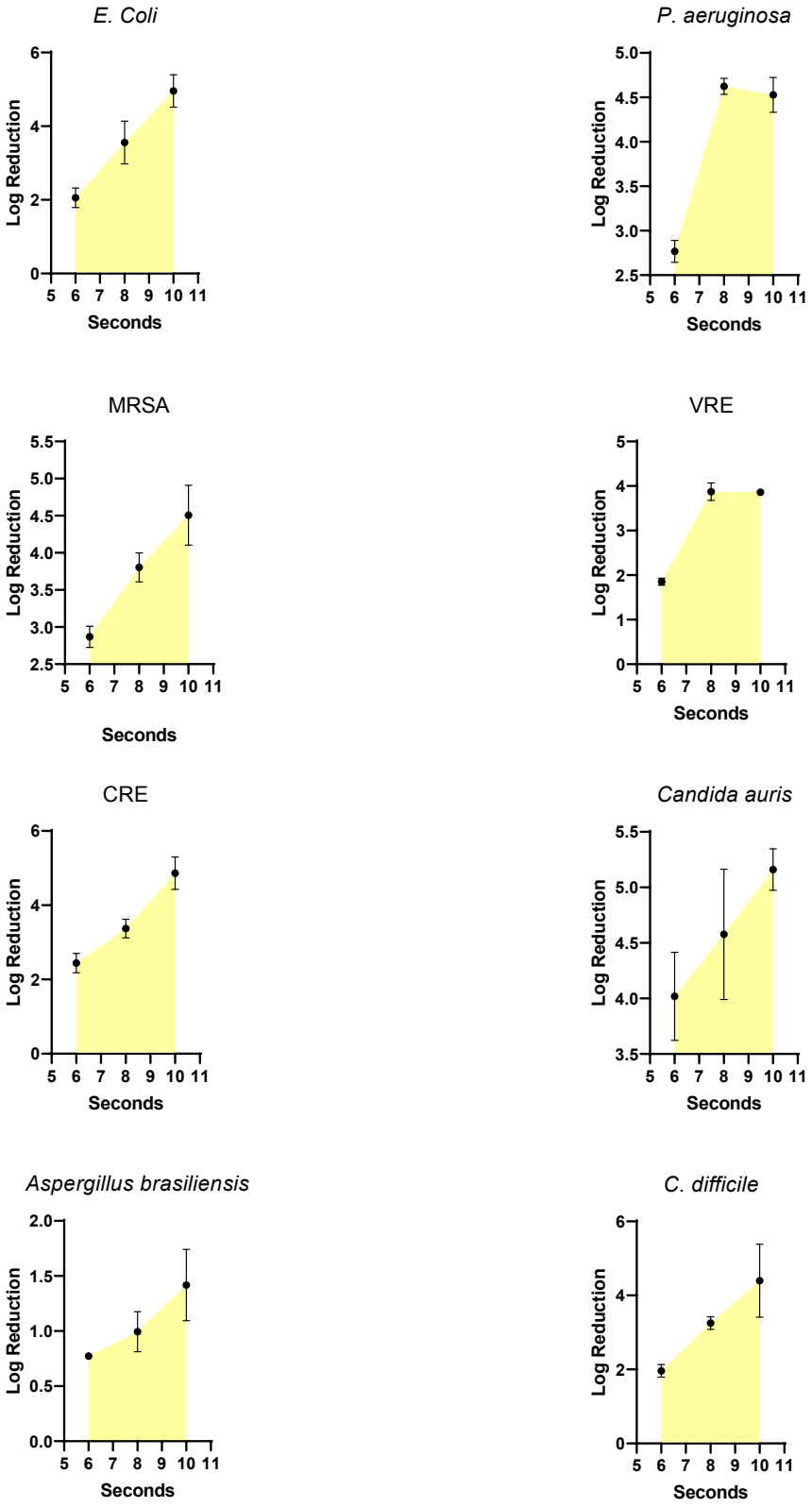


Figure 2. Individual point plots of the mean log reduction at each time point for each microorganism tested.

Written NSF approval is required for reproduction of this report. Only authorized reports in their entirety may be distributed. This report does not represent authorization to use the NSF Mark. NSF Certification may be confirmed at www.nsf.org. The results of this report relate only to those items tested.

NSF International – Applied Research Center
789 N. Dixboro Rd. Ann Arbor, MI 48015, USA
1-800.NSF.MARK | +1-734.769.8010 | www.nsf.org

Table 10. Linear regression analysis results assessing the relationship of log reduction by time. The slopes for each line are significantly different from zero.

	E. Coli	*P. aeruginosa*	MRSA	VRE	CRE	*Candida auris*	*Aspergillus brasiliensis*	*C. difficile*
Best-fit values								
Slope	0.7242	0.4403	0.409	0.5031	0.605	0.2854	0.1612	0.6087
Y-intercept	-2.268	0.4502	0.4534	-0.8288	-1.281	2.303	-0.2276	-1.664
X-intercept	3.132	-1.023	-1.109	1.647	2.117	-8.067	1.412	2.734
1/slope	1.381	2.271	2.445	1.988	1.653	3.503	6.204	1.643
Std. Error								
Slope	0.08503	0.1099	0.05277	0.1138	0.06902	0.0799	0.04195	0.111
Y-intercept	0.6942	0.8973	0.4309	0.929	0.5635	0.6524	0.3425	0.9065
95% Confidence Intervals								
Slope	0.5231 to 0.9253	0.1804 to 0.7002	0.2842 to 0.5338	0.2340 to 0.7721	0.4418 to 0.7682	0.09649 to 0.4744	0.06200 to 0.2604	0.3462 to 0.8712
Y-intercept	-3.910 to -0.6265	-1.672 to 2.572	-0.5654 to 1.472	-3.025 to 1.368	-2.613 to 0.05183	0.7600 to 3.845	-1.038 to 0.5822	-3.808 to 0.4792
X-intercept	1.187 to 4.264	-14.10 to 2.413	-5.151 to 1.065	-5.766 to 3.972	-0.1165 to 3.427	-39.58 to -1.613	-9.251 to 4.045	-1.366 to 4.428
Goodness of Fit								
R square	0.912	0.6963	0.8956	0.7364	0.9165	0.6458	0.6784	0.8111
Sy.x	0.4165	0.5384	0.2585	0.5574	0.3381	0.3914	0.2055	0.5439
Is slope significantly non-zero?								
F	72.54	16.05	60.08	19.55	76.83	12.76	14.77	30.06
DFn, DFd	1, 7	1, 7	1, 7	1, 7	1, 7	1, 7	1, 7	1, 7
P value	<0.0001	0.0051	0.0001	0.0031	<0.0001	0.0091	0.0064	0.0009
Deviation from zero?	Significant	Significant	Significant	Significant	Significant	Significant	Significant	Significant
Equation	Y = 0.7242*X - 2.268	Y = 0.4403*X + 0.4502	Y = 0.4090*X + 0.4534	Y = 0.5031*X - 0.8288	Y = 0.6050*X - 1.281	Y = 0.2854*X + 2.303	Y = 0.1612*X - 0.2276	Y = 0.6087*X - 1.664

Written NSF approval is required for reproduction of this report. Only authorized reports in their entirety may be distributed. This report does not represent authorization to use the NSF Mark. NSF Certification may be confirmed at www.nsf.org. The results of this report relate only to those items tested.

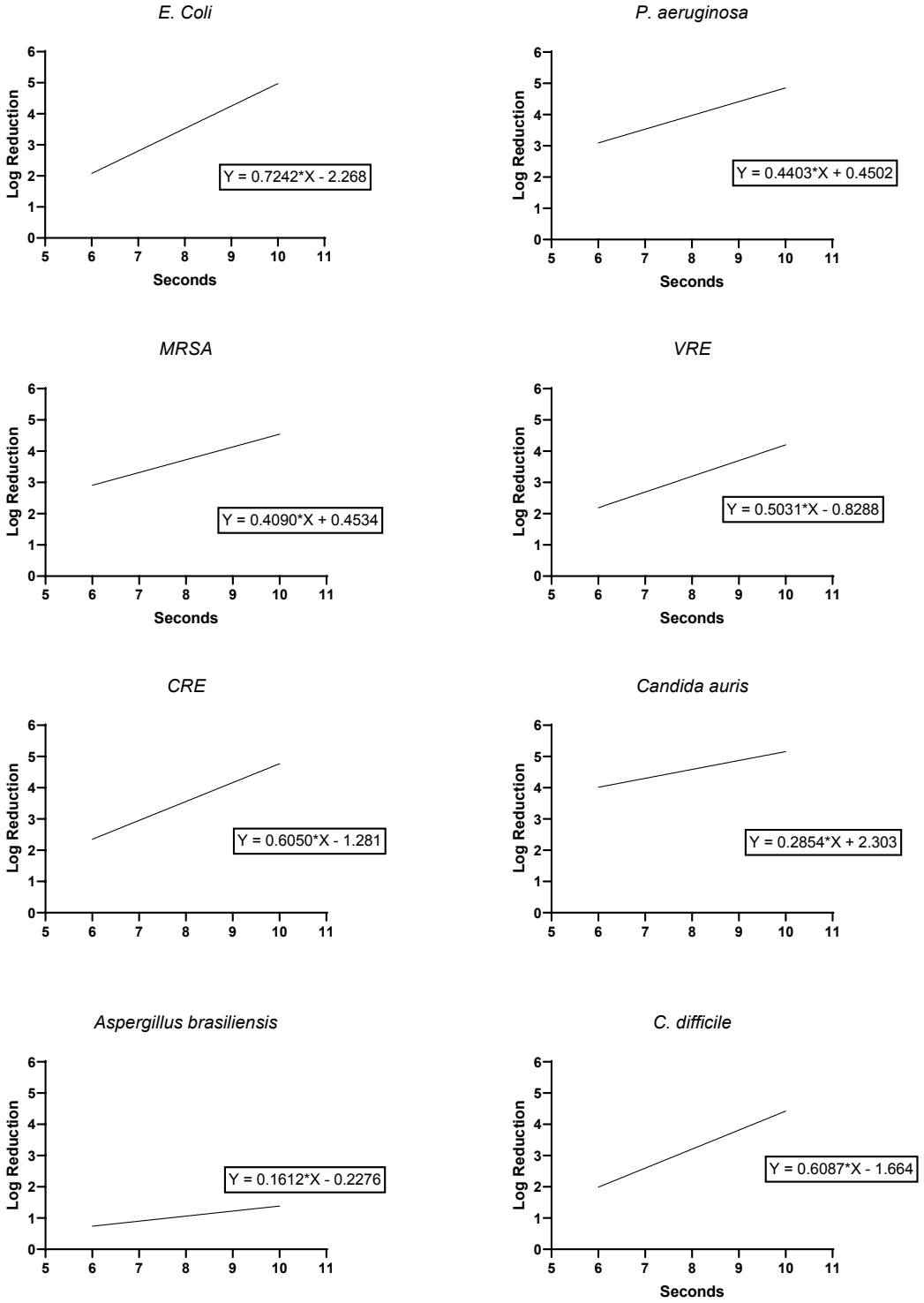


Figure 3. Regression analysis plot for each microorganism. Formula for the line is presented for each plot.

Written NSF approval is required for reproduction of this report. Only authorized reports in their entirety may be distributed. This report does not represent authorization to use the NSF Mark. NSF Certification may be confirmed at www.nsf.org. The results of this report relate only to those items tested.

Testing Laboratories:

	Lab ID	Note
All work performed at:	Approved Subcontract	GLP, non-GLP compliant

Written NSF approval is required for reproduction of this report. Only authorized reports in their entirety may be distributed. This report does not represent authorization to use the NSF Mark. NSF Certification may be confirmed at www.nsf.org. The results of this report relate only to those items tested.

STUDY TITLE

Assessment of PathO3Gen Solutions™ Footwear Sanitizing Station for Decontaminating Hard, Non-Porous Environmental Surfaces: Testing against Human Respiratory Coronavirus 229E (ATCC VR-740) as a representative Healthcare-Associated Pathogen

TEST ORGANISM

Coronavirus 229E (ATCC VR-740)

TEST SAMPLE IDENTITY

PathO3Gen Solutions™ Footwear Sanitizing Station

TEST Method

Modified Quantitative Disk Carrier Test Method (ASTM 2197) to Determine the Virucidal Activity of an Ozone-Generating Whole-Shoe Disinfection Device

AUTHOR

Dr. Syed A. Sattar
Study Director

STUDY COMPLETION DATE

March 20, 2020

PERFORMING LABORATORY

CREM Co. Labs. Units 1-2, 3403 American Dr., Mississauga, Ontario, Canada L4V 1T4

SPONSOR

PathO3Gen Solutions™

STUDY NUMBER

PTGS200219-01

STUDY PERSONNEL

STUDY DIRECTOR: Syed A. Sattar, PhD

PROFESSIONAL PERSONNEL INVOLVED: Bahram Zargar, PhD
Sepideh Khoshnevis, MSC.

TEST SYSTEM

1. Test Microorganism

Coronavirus 229E (ATCC VR-740): Coronavirus 229E (ATCC VR-740) is an enveloped virus in the genus Coronavirus. Members of this genus can cause acute respiratory infections such as SARS-1 and SARS-2 (19-nCOV). Unlike Coronavirus 229E, SARS-1, SARS-2 and Middle-East Respiratory Syndrome (MERS) virus require Biosafety Level 3 labs. Therefore, Coronavirus 229E is frequently used as surrogate for them to assess the activity of different technologies for infection prevention and control (IPAC).

2. Host Cell Line

L-132 cells were used as hosts to support the replication and quantitation of 229E.

The cells were seeded into 12-well multi-well cell culture plates containing modified Eagle's medium (MEM) supplemented with 10% fetal bovine serum (FBS) and maintained at 36±1°C in a humidified atmosphere of 5% CO_2. Efficacy test was performed when the cell monolayer reached >90% confluency.

3. Preparation of Test Inocula

To prepare the virus for inoculation, the virus stock was mixed directly with the soil load (5% FBS). Dilution of the mixture was prepared using Earle's balanced salt solution (EBSS; pH 7.2-7.4).

.

TEST METHOD

1. **Preparation of Test Substance**

The efficacy tests were performed on PathO3Gen Solutions™ Footwear Sanitizing Station following the instruction in the device's user manual at three exposure times (6, 8 and 10 seconds).

2. Test Procedure

A quantitative test system to closely simulate the field-application of the environmental surface decontamination process (modified quantitative carrier test – Tier 2 or QCT-2 (ASTM 2197)) was applied. The protocol was adapted to test the UV LED-based technology. Disks (2 cm diameter) from croc sole shoe were used as archetypical environmental surfaces. Sterile disks were placed on a small platform which was the same size of a shoe at three different positions (middle, back and front). The platform was taped at the bottom of the shoe. The platforms with the disks were exposed to the UV without touching the glass cover of the device. The disks were retrieved in an eluent/neutralizer immediately at the end of the exposure time. The disks were then eluted and the eluates assayed for viable virus.

Each disk on the platform was contaminated with 20 µL of the virus inoculum with a soil load (5% FBS) and left to dry (contaminated platform) under an operating biosafety cabinet (BSC) for 30±10 minutes. Three disks were contaminated and used as controls.

Experimental Design

a) Input

The stock virus utilized in the testing was titrated by 10-fold serial dilutions and plaque assayed for infectivity to determine the starting titer of the virus. The results of this control were for informational purposes only.

b) Cytotoxicity Control

Prior to the test, cytotoxicity control and control for interference with virus infectivity were performed to determine if the shoe material caused any apparent degeneration (cytotoxicity) of the host cell line. Control monolayers received an equivalent volume of EBSS (without any neutralizer) only.

c) Efficacy Test

1. Disks (2 cm diameter) from croc sole shoes were used in testing of this method, 3 disks were assessed as control without exposure to UV.
2. Disks were left inside an operating BSC to dry.
3. Disks were inserted on a platform with the same size of the shoe at three different locations (front, middle and back).
4. The platform was taped to the bottom of a shoe.
5. The experimenter put on the shoes with the platforms at their bottom.
6. The experimenter stepped on the device which was already on for 10 minutes.
7. After the specific exposure time, the experimenters stepped out of the device.
8. The disks were removed from the platform and each disk was placed into a Nalgene vial containing 2 mL of an eluent.
9. The L-132 cells in multi-well culture plates were inoculated with 100 µL of the dilutions prepared from test and control samples. Uninfected indicator cell cultures (cell controls) were inoculated with 100 µL EBSS alone. The cultures were incubated at 33±1°C in a humidified atmosphere of 5% CO_2 for 40-44 hrs before fixing and staining them for counting the plaque-forming units (PFU).

10. Three control disks were included in each test to estimate the initial contamination on the platform. The test was initiated with processing one control before the processing test carriers, one in the middle of the test and ended up with the third control. This was done to take into the account the changes in the input level of the test organisms during the experiment.

DATA ANALYSIS

Calculation of Log_{10} Reduction

Log_{10} Reduction = Log_{10} of average PFU from control carriers – log_{10} of average PFU the test carriers.

STUDY ACCEPTANCE CRITERIA

No product acceptance criterion was specified for this range-finding study.

TEST RESULTS

The initial challenge on each carrier were 3.68, 3.73 and 3.65 log_{10} PFU in three different tests performed on PathO3Gen Solutions™ Footwear Sanitizing Station. Table 1 show the result of log_{10} reduction for each contact time. In this test, the drying time of inoculated disks was reduced to 1 hr. In all contact times the log_{10} reduction was more than 3. No plaque was detected for 8 and 10 seconds contact times.

Table 1: Virucidal Activity Test of PathO3Gen Solutions™ Footwear Sanitizing Station against Coronavirus 229E (ATCC VR-740) with three different contact times

Contact times	**Log_{10} Reduction in PFU**			
	Test #1	**Test #2**	**Test #3**	**Average of Three tests**
6 seconds	3.07	3.28	3.42	3.27
8 seconds	3.68	3.73	3.65	3.69
10 seconds	3.68	3.73	3.65	3.69

APPENDIX

Result of efficacy test on the device with three different contact times (6, 8 and 10 seconds) against Coronavirus 229E dried on carriers representing shoe soles.

Test #1												
Contact Time	6 seconds			8 seconds			10 seconds			Control		
Dilution	Front	Middle	Back	Front	Middle	Back	Front	Middle	Back	C1	C2	C3
10^{-0}	0,1,0	1,1,0	0,1,1	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	TNTC	TNTC	TNTC
10^{-1}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	21,16,25	25,24,25	11,16,23
10^{-2}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	3,3,3	2,2,2	2,2,2
10^{-3}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0
10^{-4}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0

C= Control
TNTC= Too numerous to count

Test #2												
Contact Time	6 seconds			8 seconds			10 seconds			Control		
Dilution	Front	Middle	Back	Front	Middle	Back	Front	Middle	Back	C1	C2	C3
10^{-0}	0,1,1	1,0,0	1,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	TNTC	TNTC	TNTC
10^{-1}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	24,25,25	21,22,24	24,20,25
10^{-2}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	4,3,2	3,2,4	3,3,4
10^{-3}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0
10^{-4}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0
Test #3												
Contact Time	6 seconds			8 seconds			10 seconds			Control		
Dilution	Front	Middle	Back	Front	Middle	Back	Front	Middle	Back	C1	C2	C3
10^{-0}	0,0,0	0,1,0	0,1,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	TNTC	TNTC	TNTC
10^{-1}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	20,22,22	25,25,17	21,19,19
10^{-2}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	2,2,2	2,2,4	3,2,2
10^{-3}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0
10^{-4}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0

The use of the CREM Co. Labs' name, logo or any other representation of CREM Co. Labs without the written approval of CREM Co., Inc. is prohibited. In addition, CREM Co Labs may not be referred to any form of promotional materials, press release, advertising or similar materials (whether by print, broadcast, communication or electronic means) without the expressed written permission of CREM Co., Inc.

STUDY TITLE

Assessment of PathO3Gen Solutions™ Footwear Sanitizing Station for Decontaminating Hard, Non-Porous Environmental Surfaces: Testing against Murine Norovirus (Strain S99) as a representative Healthcare-Associated Pathogen

TEST ORGANISM

Murine Norovirus (Strain S99)

TEST SAMPLE IDENTITY

PathO3Gen Solutions™ Footwear Sanitizing Station

TEST Method

Modified Quantitative Disk Carrier Test Method (ASTM 2197) to Determine the Virucidal Activity of an Ozone-Generating Whole-Shoe Disinfection Device

AUTHOR

Dr. Syed A. Sattar
Study Director

STUDY COMPLETION DATE

Feb 22, 2021

PERFORMING LABORATORY

CREM Co. Labs. Units 1-2, 3403 American Dr., Mississauga, Ontario, Canada L4V 1T4

SPONSOR

PathO3Gen Solutions™

STUDY NUMBER

PTGS200219-02

STUDY PERSONNEL

STUDY DIRECTOR: Syed A. Sattar, PhD

PROFESSIONAL PERSONNEL INVOLVED: Bahram Zargar, PhD
Golnoosh Sedghroohi, PhD
Edwardo Suarez, PhD.

TEST SYSTEM

1. Test Microorganism

Murine Norovirus (MNV): MNV is a non-enveloped RNA virus in the family Caliciviridae. It is the most prevalent viral infection in mice. There are 4 described strains designated MNV-1, MNV-2, MNV-3, and MNV-4, as well as multiple field strains. The virus causes enteric infections and can also exit the gut to replicate in macrophages and dendritic cells in multiple organs, including mesenteric lymph nodes and liver.

Since human noroviruses are difficult to culture in the lab, MNV is frequently used as its surrogate.

2. Host Cell Line

RAW 264.7 cells were used as host cell to support replication of MNV. This cell line is a macrophage-like, Abelson leukemia virus transformed cell line derived from BALB/c mice. This cell line is a commonly used model of mouse macrophages for the study of efficacy test of disinfectants.

The cells were seeded into 12-well cell culture plates containing modified Eagle's minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS) and maintained at 36±1°C in a humidified atmosphere of 5% CO_2. Efficacy test was performed when the cell monolayer reached >90% confluency.

3. Preparation of Test Inocula

To prepare the virus for inoculation, the virus stock was mixed directly with the soil load (5% FBS). Dilution of the mixture was prepared using Earle's balanced salt solution (EBSS; pH 7.2-7.4).

.

TEST METHOD

1. Preparation of Test Substance

The efficacy tests were performed on PathO3Gen Solutions™ Footwear Sanitizing Station following the instruction in the device's user manual at three exposure times (6, 8 and 10 seconds).

2. Test Procedure

A quantitative test system to closely simulate the field-application of the environmental surface decontamination process (modified quantitative carrier test – Tier 2 or QCT-2 (ASTM 2197)) was applied. The protocol was adapted to test the UV LED-based technology. Disks (2 cm diameter) from croc sole shoe were used as archetypical environmental surfaces. Sterile disks were placed on a small platform which was the same size as a shoe at three different positions (middle, back and front). The platform was taped at the bottom of the shoe. The platforms with the disks were exposed to the UV without touching the glass cover of the device. The disks were retrieved in an eluent/neutralizer immediately at the end of the exposure time. The disks were then eluted and the eluates

assayed for viable virus.

Each disk on the platform was contaminated with 20 µL of the virus inoculum with a soil load (5% FBS) and left to dry (contaminated platform) under an operating biosafety cabinet (BSC) for 30±10 minutes. Three disks were contaminated and used as controls.

Experimental Design

a) Input

The stock virus utilized in the testing was titrated by 10-fold serial dilutions and plaque assayed for infectivity to determine the starting titer of the virus. The results of this control were for informational purposes only.

b) Efficacy Test

1. Disks (2 cm diameter) from crocs sole shoes were used in testing of this method, 3 disks were assessed as control without exposure to UV.
2. Disks were left inside an operating BSC to dry.
3. Disks were inserted on a platform with the same size of the shoe at three different locations (front, middle and back).
4. The platform was taped to the bottom of a shoe.
5. The experimenter put on the shoes with the platforms at their bottom.
6. The experimenter stepped on the device which was already on for 10 minutes.
7. After the specific exposure time, the experimenters stepped out of the device.
8. The disks were removed from the platform and each disk was inserted into a Nalgene vial containing 2 mL of an eluent.
9. The RAW 264.7 cells in multi-well culture plates were inoculated with 100 µL of the dilutions prepared from test and control samples. Uninfected indicator cell cultures (cell controls) were inoculated with 100 µL EBSS alone. The cultures were incubated at 36±1°C in a humidified atmosphere of 5% CO_2 for 44-48 hrs before fixing and staining them for counting the plaque-forming units (PFU).

10. Three control disks were included in each test to estimate the initial contamination on the platform. The test was initiated with processing one control before the processing test carriers, one in the middle of the test and ended up with the third control. This was done to take into the account the changes in the input level of the test organisms during the experiment.

DATA ANALYSIS

Calculation of Log_{10} Reduction

Log_{10} Reduction = Log_{10} of average PFU from control carriers – log_{10} of average PFU the test carriers.

STUDY ACCEPTANCE CRITERIA

No product acceptance criterion was specified for this range-finding study.

TEST RESULTS

The initial levels of challenge on each carrier were 4.15, 4.36 and 4.41 log_{10} PFU in three different tests performed on PathO3Gen Solutions™ Footwear Sanitizing Station. Table 1 show the result of log_{10} reductions for each contact time. In this test, the drying time of inoculated disks was reduced to 1 hr. In all contact times the log10 reduction was more than 4.31. No plaque was detected for 6, 8 and 10 seconds contact times.

Table 1: Virucidal Efficacy Test of PathO3Gen Solutions™ Footwear Sanitizing Station against Murine Norovirus (Strain S99) with three different contact times

Contact times	Log Reduction in PFU			
	Test #1	Test #2	Test #3	Average of Three tests
6 seconds	>4.15	>4.36	>4.41	>4.31
8 seconds	>4.15	>4.36	>4.41	>4.31
10 seconds	>4.15	>4.36	>4.41	>4.31

APPENDIX

Result of efficacy test on the device with three different contact times (6, 8 and 10 seconds) against Murine Norovirus (Strain S99) dried on carriers representing shoe soles.

Test #1												
Contact Time	6 seconds			8 seconds			10 seconds			Control		
Dilution	Front	Middle	Back	Front	Middle	Back	Front	Middle	Back	C1	C2	C3
10^{-0}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	TNTC	TNTC	TNTC
10^{-1}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	40,40,39	34,28,32	TNTC
10^{-2}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	3,2,3	4,5,4	29,31,24
10^{-3}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	2,1,2	2,3,2
10^{-4}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0

C= Control
TNTC= Too numerous to count

Test #2												
Contact Time	6 seconds			8 seconds			10 seconds			Control		
Dilution	Front	Middle	Back	Front	Middle	Back	Front	Middle	Back	C1	C2	C3
10^{-0}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	TNTC	TNTC	TNTC
10^{-1}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	TNTC	TNTC	TNTC
10^{-2}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	12,11,12	10,10,9	12,11,11
10^{-3}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	2,1,2	2,1,2	2,1,2
10^{-4}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0
Test #3												
Contact Time	6 seconds			8 seconds			10 seconds			Control		
Dilution	Front	Middle	Back	Front	Middle	Back	Front	Middle	Back	C1	C2	C3
10^{-0}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	TNTC	TNTC	TNTC
10^{-1}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	TNTC	TNTC	TNTC
10^{-2}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	13,10,10	11,8,10	10,11,12
10^{-3}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	3,2,4	1,1,1	1,1,1
10^{-4}	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0

The use of the CREM Co. Labs' name, logo or any other representation of CREM Co. Labs without the written approval of CREM Co., Inc. is prohibited. In addition, CREM Co Labs may not be referred to in any form of promotional materials, press release, advertising or similar materials (whether by print, broadcast, communication or electronic means) without the expressed written permission of CREM Co., Inc.

MICROCHEM LABORATORY

STUDY REPORT

Study Title

Antibacterial Activity and Efficacy of Patho$_3$gen Solutions' UVZone Shoe Sanitizing Station Device

Test Method

Custom Device Study Based on: Modified ASTM E1153

Study Identification Number

NG19887

Study Sponsor

Scott Beal
Patho$_3$gen Solutions
scott@patho3gen.com
(502) 930-6526

Test Facility

Microchem Laboratory
1304 W. Industrial Blvd
Round Rock, TX 78681
(512) 310-8378
Testing performed by: Kyra Christensen

ASTM E1153: General Information

ASTM International, formerly the American Society for Testing and Materials (ASTM), is an internationally recognized organization that develops and publishes product and testing standards. ASTM E1153 is a quantitative test method designed to evaluate the antimicrobial efficacy of sanitizers on pre-cleaned inanimate, nonporous, non-food contact surfaces. The method is typically used with a maximum contact time of 5 minutes, during which the sanitizer reduces the concentration of viable test microorganisms. ASTM E1153 utilizes non-antimicrobial agents as controls to establish baselines for microbial reductions. The ASTM E1153 method is a benchmark method for non-food contact surface sanitizers and is recognized by several regulatory agencies as an approved method for claim substantiation. See study modifications for changes made to the study method to accommodate a device.

Laboratory Qualifications Specific to ASTM E1153

Microchem Laboratory began conducting the ASTM E1153 test method in 2007. Since then, the laboratory has performed hundreds of ASTM E1153 tests on a broad array of test substances, against a myriad of bacterial and fungal species. The laboratory is also experienced with regard to modifying the test method as needed in order to accommodate customer needs. Every ASTM E1153 test at Microchem Laboratory is performed in a manner appropriate for the test substances submitted by the Study Sponsor, while maintaining the integrity of the method.

Study Timeline

Test Device Received	Cultures Initiated	Treatment	Enumeration Plates Incubated	Enumeration Plates Evaluated	Report Delivered
08JUL2022	20JUL2022	21JUL2022	21JUL2022	25JUL2022	26JUL2022

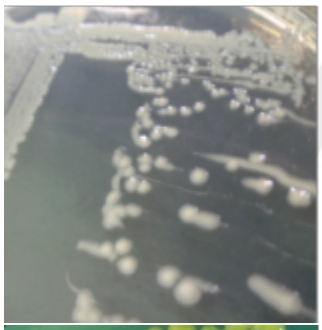
1304 W. INDUSTRIAL BLVD ROUND ROCK, TX 78681 | CONTACT@MICROCHEMLAB.COM | 512.310.8378

Test Device Information

Name of Test Device: UVZone Shoe Sanitizing Station (received 08JUL2022)
Manufacturer: Patho$_3$gen Solutions
Mode of Disinfection: UVC and Ozone

Test Microorganism Information

The test microorganism(s)selected for this test:

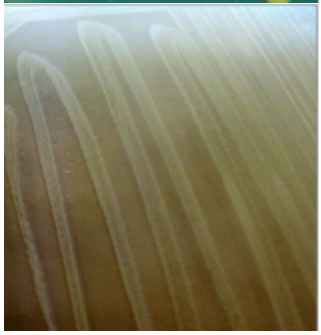


Salmonella enterica

This bacteria is Gram-negative, rod-shaped, facultative anaerobe. Like the closely related *Escherichia* genus, *Salmonella* are common to all parts of the world and share habitats in the digestive systems of cold and warm-blooded animals. *S. enterica* is one of the most common bacteria associated with zoonotic and foodbourne illness. Because of it's regular occurrence and pathogenicity, *S. enterica* is a common bacteria for measuring disinfectant efficacy.

***Cronobacter sakazakii* ATCC 29004**

This bacteria is a Gram-negative, rod-shaped, pathogenic bacterium that can live in very dry places. It is described as a ubiquitous and opportunistic pathogen that currently contaminates a wide spectrum of foods and poses a lethal threat to neonates, the elderly, and persons with immune deficiencies.

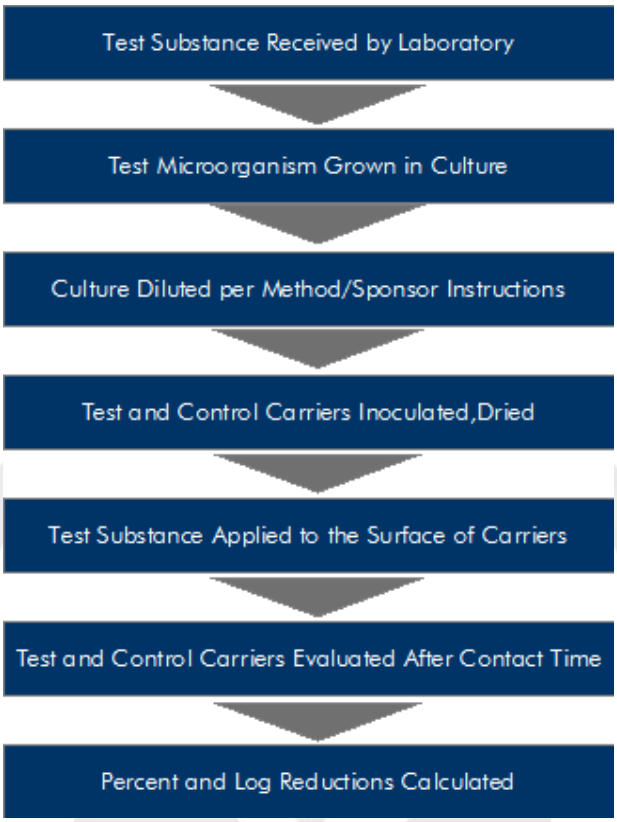


Listeria monocytogenes

This bacteria is a Gram-positive, rod shaped, facultative anaerobe that is motile due to the presence of flagella. These bacteria are common cause of the foodbourne illness listeriosis, which can be fatal. Listeriosis can cause meningitis and sepsis and is particularly dangerous to pregnant women and unborn infants. *Listeria monocytogenes* is pervasive and can be found in soil, water, and certain livestock animals. They can resist both heat and freezing and can survive for several years.

1304 W. INDUSTRIAL BLVD. ROUND ROCK, TX 78681 | CONTACT@MICROCHEMLAB.COM | 512.310.8378

Diagram of the Procedure



Summary of the Procedure

- The test microorganisms were prepared in liquid culture.
- Sterilized carriers were inoculated with 0.20 mL of the test cultures. Inoculated carriers were allowed to dry completely prior to testing.
- Control carriers were harvested prior to testing.
- Test carriers were treated with the device for 6, 8, and 10 seconds.
- At the conclusion of the contact time, test carriers were harvested.
- Dilutions of the harvested carriers were enumerated to determine the surviving microorganisms at the respective contact times.
- To determine log and percent reductions, the microbial concentrations of the carriers treated by the test device were compared to the that of the untreated parallel controls.

1304 W. INDUSTRIAL BLVD. ROUND ROCK, TX 78681 | CONTACT@MICROCHEMLAB.COM | 512.310.8378

Criteria for Scientific Defensibility of a Custom Device Study

For Microchem Laboratory to consider a Device Study to be scientifically defensible, the following criteria must be met:

1. The average number of viable bacteria recovered from the time zero samples must be approximately 1 x 10^5 CFU/carrier or greater.
2. Positive/Growth controls must demonstrate growth of the appropriate test microorganism.
3. Negative/Purity controls must demonstrate no growth of test microorganism.

Passing Criteria

Due to the modified nature of the study, passing criteria may be determined by the Study Sponsor.

Testing Parameters

Carriers (Size):	1" x 3" Glass Slides		**Replicates:**	Triple
Culture Growth Media:	Tryptic Soy Broth, Nutrient Broth for CS29004		**Culture Growth Time:**	18-24 hours
Culture Dilution Media:	Phosphate Buffered Saline		**Inoculum Volume:**	0.20 mL
Inoculum Concentration:	Approx. 1.0 x 10^7 CFU/Carrier		**Contact Temperature:**	Ambient
Contact Time:	6, 8 and 10 seconds		**Enumeration Media:**	Tryptic Soy Agar (TSA), Nutrient Agar (NTA) for CS29004
Neutralizer (Vol.):	Phosphate Buffered Saline (PBS) w/ 0.1% Tween 80		**Enumeration Plate Incubation Time:**	24-48 hours
Enumeration Plate Incubation Temperature:	36°C ± 1°C, 30°C ± 1°C for CS29004			

1304 W. INDUSTRIAL BLVD. ROUND ROCK, TX 78681 | CONTACT@MICROCHEMLAB.COM | 512.310.8378

Study Notes

The glass carriers were placed inverted for the microorganisms to be in contact with the device's surface, to simulate the sole of a shoe.

The device was disinfected with ethanol and allowed to dry in between each test run.

1304 W. INDUSTRIAL BLVD. ROUND ROCK, TX 78681 | CONTACT@MICROCHEMLAB.COM | 512.310.8378

Control Results

Neutralization Method:	N/A	Media Sterility:	Confirmed
Growth Confirmation:	Confirmed		

Calculations

$$\text{Percent Reduction} = \left(\frac{B - A}{B}\right) \times 100$$

Where:

B = Number of viable test microorganisms on the control carriers immediately after inoculation

A = Number of viable test microorganisms on the test carriers after the contact time

$$\text{Log}_{10}\text{Reduction} = \text{Log}\left(\frac{B}{A}\right)$$

Where:

B = Number of viable test microorganisms on the control carriers immediately after inoculation

A = Number of viable test microorganisms on the test carriers after the contact time

1304 W. INDUSTRIAL BLVD. ROUND ROCK, TX 78681 | CONTACT@MICROCHEMLAB.COM | 512.310.8378

Results of the Study

Table 1: Results for *Cronobacter sakazakii*

Test Microorganism	Device	Contact Time	Replicate	CFU/Carrier	Average CFU/Carrier	Average Percent Reduction Compared to Parallel Controls	Average Log10 Reduction Compared to Parallel Controls
Cronobacter sakazakii ATCC 29004	N/A	Time Zero	Control 1	1.12E+05	1.10E+05	N/A	
			Control 2	7.50E+04			
			Control 3	1.44E+05			
	UVZone Shoe Sanitizing Station	6 seconds	Replicate 1	<1.00E+01	<1.00E+01	>99.991%	>4.04
			Replicate 2	<1.00E+01			
			Replicate 3	<1.00E+01			
		8 seconds	Replicate 1	1.00E+01	<3.67E+01	>99.97%	>3.48
			Replicate 2	9.00E+01			
			Replicate 3	<1.00E+01			
		10 seconds	Replicate 1	<1.00E+01	<1.00E+01	>99.991%	>4.04
			Replicate 2	1.00E+01			
			Replicate 3	<1.00E+01			
The limit of detection for this assay was 1.00E+01 CFU/carrier. For all values below this amount, the value is recorded as <1.00E+01.							

Table 2: Results for *Salmonella enterica*

Test Microorganism	Device	Contact Time	Replicate	CFU/Carrier	Average CFU/Carrier	Average Percent Reduction Compared to Parallel Controls	Average Log10 Reduction Compared to Parallel Controls
Salmonella enterica ATCC 10708	N/A	Time Zero	Control 1	3.30E+05	6.39E+05	N/A	
			Control 2	2.06E+05			
			Control 3	1.38E+06			
	UVZone Shoe Sanitizing Station	6 seconds	Replicate 1	<1.00E+01	<1.00E+01	>99.998%	>4.81
			Replicate 2	<1.00E+01			
			Replicate 3	<1.00E+01			
		8 seconds	Replicate 1	<1.00E+01	<1.67E+01	>99.997%	>4.58
			Replicate 2	<1.00E+01			
			Replicate 3	3.00E+01			
		10 seconds	Replicate 1	3.50E+02	<1.23E+02	>99.98%	>3.71
			Replicate 2	<1.00E+01			
			Replicate 3	<1.00E+01			
The limit of detection for this assay was 1.00E+01 CFU/carrier. For all values below this amount, the value is recorded as <1.00E+01.							

1304 W. INDUSTRIAL BLVD. ROUND ROCK, TX 78681 | CONTACT@MICROCHEMLAB.COM | 512.310.8378

Results of the Study continued

Table 3: Results for *Listeria monocytogenes*

Test Microorganism	Device	Contact Time	Replicate	CFU/Carrier	Average CFU/Carrier	Average Percent Reduction Compared to Parallel Controls	Average Log10 Reduction Compared to Parallel Controls
Listeria monocytogenes ATCC 19115	N/A	Time Zero	Control 1	1.07E+05	1.62E+05	N/A	
			Control 2	2.06E+05			
			Control 3	1.73E+05			
	UVZone Shoe Sanitizing Station	6 seconds	Replicate 1	2.30E+02	<8.33E+01	>99.95%	>3.29
			Replicate 2	<1.00E+01			
			Replicate 3	<1.00E+01			
		8 seconds	Replicate 1	<1.00E+01	<1.00E+01	>99.994%	>4.21
			Replicate 2	<1.00E+01			
			Replicate 3	<1.00E+01			
		10 seconds	Replicate 1	<1.00E+01	<1.67E+01	>99.9897%	>3.99
			Replicate 2	<1.00E+01			
			Replicate 3	3.00E+01			
The limit of detection for this assay was 1.00E+01 CFU/carrier. For all values below this amount, the value is recorded as <1.00E+01.							

The results of this study apply to the tested substances(s) only. Extrapolation of findings to related materials is the responsibility of the Sponsor.

Copyright © Microchem Laboratory, 2018. Reproduction and ordinary use of this study report by the entity listed as "Sponsor" is permitted. Other copying and reproduction of all or part of this document by other entities is expressly prohibited, unless prior permission is granted in writing by Microchem Laboratory.

1304 W. INDUSTRIAL BLVD. ROUND ROCK, TX 78681 | CONTACT@MICROCHEMLAB.COM | 512.310.8378